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ARC-Vegetable, Industrial and Medicinal Plants Newsletter

Newsletter of the Vegetable, Industrial and Medicinal Plants, campus in the Crop Sciences Programme of the Agricultural Research Council (ARC)

Development of a new method for fingerprinting potato cultivars in South Africa using SNPs

Compiled by Dr Inge Gazendam

Potato is one of the four major staple crops in the world, and the largest vegetable commodity in South Africa. More than 4000 varieties of potato are grown worldwide in more than 150 countries. Trueness-to type information of cultivars is essential for seed potato growers to provide true varieties to the potato production industry. DNA fingerprinting is a molecular technique applied to identify genetic differences between cultivars or lines (clonal identity) and used for genetic purity testing (trueness-to-type). The advantage of potato DNA fingerprinting is that it can be done at very early developmental stages, such as on mini-tubers or *in vitro* leaf material (Fig. 1), and is less resource intensive than morphological methods. Accidental mix-ups can therefore be identified at an early stage, before *in vitro* multiplication, to prevent costly mistakes later on. Cultivar genetic identity is important in the protection of plant breeders' rights. The *in vitro* laboratory of the ARC-VIMP is also dependent on DNA fingerprinting, and cannot release material to the industry unless trueness-to-type is confirmed.

The DNA fingerprinting method that has been applied at the ARC-VIMP until now is based on the polymerase chain reaction (PCR) of simple sequence repeat (SSR) markers, and the resulting mixtures of DNA fragments are separated according to their size by denaturing polyacrylamide gel electrophoresis (PAGE) on a large gel system. The fingerprint fragments are then stained and scored for their presence and size (Fig. 2). Differences are observed as the presence or absence of a particular fragment. The results are also visually compared with a known reference cultivar fingerprint. This outdated method has a few disadvantages, namely, being low throughput, labour intensive, incurs a high cost per data point, and scoring is highly subjective due to the indirect method of determining fragment sizes. The equipment necessary to perform the technique (gel comb) and reagents to size the SSR alleles (10 bp ladder) have also been discontinued.

Single nucleotide polymorphisms (SNP) became the marker of choice for applications in plant breeding and genetics because they are more abundant, stable, amenable to automation, efficient, and increasingly cost-effective. A SNP is the variation in a single nucleotide that occurs at a specific position in the genome of any organism. SNPs are highly abundant in plants and are spread out evenly over the genome. In the potato genome large numbers of SNPs have been identified and one SNP is found on average in every 20 base pairs (bp). SNP genotyping is therefore suggested as the most modern method for variety identification.

Figure 1. Potato DNA fingerprinting can easily be done from different early stage starting material.

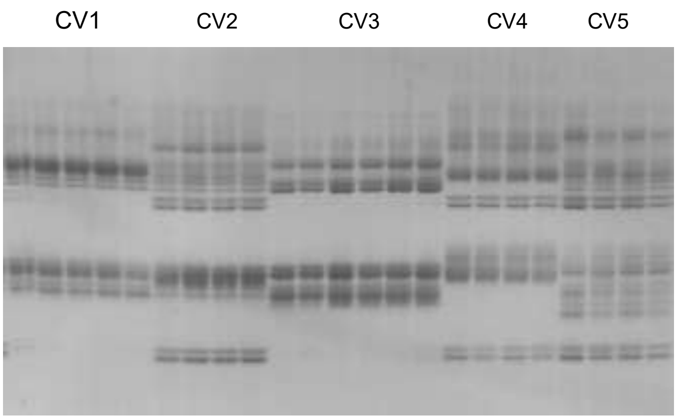

Figure 2. DNA fragments generated by PCR of SSR sequences, separated on a large PAGE gel and stained with silver staining. The SSR fingerprints of five cultivars, indicated by CV 1 to 5, are presented for the same SSR marker.

Phasing out the old SSR and polyacrylamide gel electrophoresis (PAGE) method for DNA fingerprinting required protocol development to migrate to SNP genotyping. The aim is to better serve the South African potato industry through the deployment of an efficient, reliable and cost effective genetic fingerprinting method for clonal identification and trueness-to-type determination of potato cultivars. Funding for protocol development was secured from Potatoes South Africa.

Most of the cultivated potato cultivars have four copies of each chromosome. A specific potato cultivar can therefore have one of five SNP genotypes, indicated by the number of times one of the SNP alleles occurs, also called the dosage number. For example, if “A” and “B” are the different alleles of a given SNP and “A” is the reference allele, then the allele dosage classes are 0 (BBBB), 1 (ABBB), 2 (AABB), 3 (AAAB) or 4 (AAAA) (Fig. 3). Dissimilarity between potato varieties can be caused by one dosage difference, suggesting that allele dosage is useful for variety identification. SNP markers are able to estimate allele dosage, therefore the nucleotide genotype, as well as the copy number can be determined in a polyploid genome. In contrast, it is nearly impossible to determine the copy number of a fragment produced using an SSR marker.

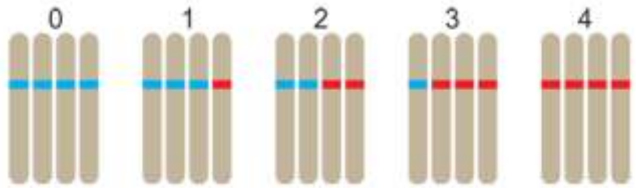

Figure 3. Dosage of alleles in tetraploid potato. In a tetraploid species, five distinct allele dosage classes are possible at a bi-allelic marker position, ranging from 0 to 4 copies of the reference allele. Here, the reference allele is coloured red, with the alternative allele coloured blue. (Adapted from Bourke et al., 2018).

Method development and validation

During method development, initially a collection of 190 potato germplasms were genotyped at 500 SNP sites using SeqSNP from LGC Genomics. All major potato varieties planted during the 2018/2019 growing season were included in this study. A custom SNP panel was developed and optimised that can discriminate between all 173 unique potato cultivars used during method development, including those currently important in South Africa (Figure 4). SNPs were

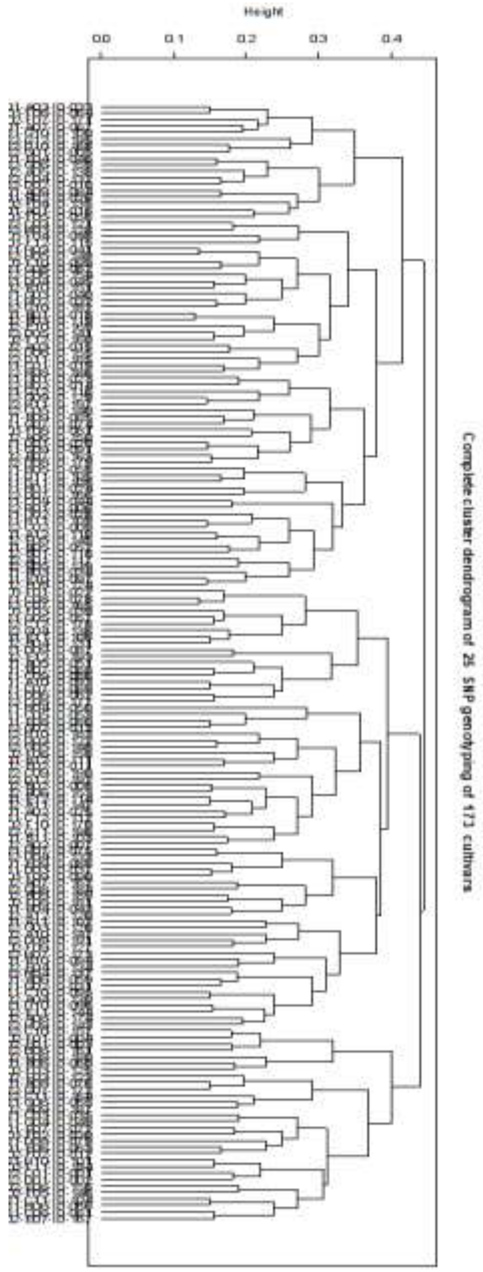

Figure 4. Dendrogram depicting the clustering patterns of 173 potato cultivars, constructed based on the pairwise genetic distances calculated with the Kosman index using the selected 25 SeqSNP markers.

carefully selected to have a balanced allele frequency, which results in a high polymorphism information content. They are therefore expected to be useful to discriminate between wider sets of potato germplasm, enabling the addition of new cultivars to the SNP genotype database. Dr I Gazendam presented on the progress at the annual forum of the Nuclear Material Producers (NUMPRO) on 18 May 2021 at the Protea Hotel Fire and Ice, Menlyn, Pretoria.

During August 2021, the SNP panel was validated with KASP (Kompetitive Allele Specific PCR) assays in the laboratory. SNP genotyping results were produced for all 25 SNP markers in the panel, and R coding was employed to analyse the data and plot the KASP results in scatter plots coloured according to the expected SeqSNP allele dosage. Only two of the 25 SNPs failed to cluster into the five dosage classes (results not shown). Two others generated crisp clusters, but a high majority of the KASP genotypes did not correlate to the expected SeqSNP genotypes (results not shown). Figure 5 illustrates how the KASP genotypes of one of the 21 remaining successful SNP markers cluster into the five dosage classes. KASP and SeqSNP genotypes corresponded perfectly, except where a small number of swopped DNA samples were discovered.

Output:
The project was successfully completed and one of the most important outputs of the project has been setting up a potato SNP genotype database (Fig. 6). The intellectual property rights regarding the identity of the SNPs in the KASP SNP panel remain the property of the ARC. SNP genotypes simplify the germplasm genotype database, enabling automatic comparisons to determine the suggested identity of an unknown cultivar.

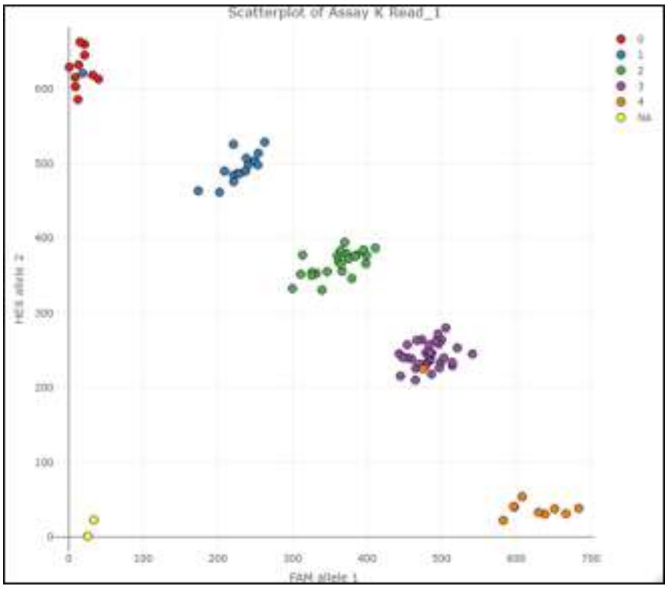

Figure 5. Scatter plot constructed with KASP data points in the colour of the expected SeqSNP allele dosage. Example of a successful KASP SNP assay using marker “K”, which was able to cluster potato genotypes into the five expected gene dosage classes. The yellow dots correspond to the water (no template) control samples. Different expected dosages (0, 1, 2, 3 or 4) are indicated by dots coloured red, blue, green, purple and orange, respectively.

Application
The SNP panel is ready for application to potato samples submitted for fingerprinting or purity requests. All private farmer customers and public or private laboratories will have access to the service. Purity tests can be performed objectively and with higher throughput using a flexible selection of the most appropriate KASP SNP assays to resolve identity or mixing issues of potato production stakeholders. The probable identity of an unknown sample can be determined by comparing the SNP genotypes of the individual to the germplasm SNP genotype database. Possible hits with the lowest genetic differences will be identified and reported to the client. If a new cultivar needs to be added to the SNP genotype database, it needs to be genotyped with all the 21 successful KASP SNP assays. It is advised to submit 12 or more samples at a time to take advantage of the reduced SNP genotyping prices compared to the current, but outdated, SSR fingerprinting prices. It is worth noting that pooling of samples does not provide a cost saving opportunity, since all genetic fingerprinting tools are based on separately assaying an individual’s genetic signature.

The SNP fingerprinting method has already been utilised to fingerprint samples for internal and external clients. During October and November 2021, the trueness-to-type of 49 samples submitted by the ARC-VIMP *in vitro* Genebank were successfully verified. During November 2021, the unknown samples submitted by an external client could successfully be correlated to cultivars in the SNP genotype database. This helped them to resolve the puzzling phenotypes of tubers sampled from the field and their factory. SNP genotyping is therefore an efficient and accurate method to determine trueness-to-type of potato for clients, and has the additional ability of clonal identification. This new method enables the ARC-VIMP to continue to serve the South African potato industry by providing a more effective and accurate genetic fingerprinting service to external and ARC-VIMP genebank clients.

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			KASP SNP assay																						
gDNA#	Cultivar name	Genotype	A	C	D	E	F	G	H	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y
5	7Four7	SeqSNP	4	0	3	3	3	0	3	1	3	3	2	2	3	1	3	2	3	2	0	3	2	2	3
5	7Four7	KASP	4	0	3	3	3	0	3	1	3	3	2	2	3	1	3	2	3	2	0	3	2	2	3
54	890/20	SeqSNP	3	1	3	1	2	1	3	3	4	2	3	4	2	1	1	2	2	2	2	2	3	3	1
86	92-0472-042	SeqSNP	1	1	1	1	2	2	3	1	1	2	2	3	2	1	1	2	1	2	0	1	2	2	3
65	92-466-112	SeqSNP	3	2	1	0	4	1	0	1	1	0	3	2	3	1	0	2	3	2	2	1	3	3	3
65	92-466-112	KASP	3	2	1	0	4	1	0	1	1	0	3	2	3	1	0	2	3	2	2	1	3	3	3
64	94-0530-008 (Freek)	SeqSNP	2	0	2	1	2	2	2	1	2	2	3	2	4	2	1	1	1	1	1	1	2	3	3
64	94-0530-008 (Freek)	KASP	2	0	2	1	2	2	2	1	2	2	3	2	4	2	1	1	1	1	1	1	2	3	3
100	95-521-126	SeqSNP	2	0	1	0	2	3	2	1	1	2	1	2	3	0	1	3	3	3	2	0	3	2	3
84	96-0568-002 (Arno)	SeqSNP	3	4	2	2	1	2	3	2	1	4	0	3	1	1	3	2	1	4	3	1	2	2	4
84	96-0568-002 (Arno)	KASP	3	4	2	2	1	2	3	2	1	4	0	3	1	1	3	2	1	4	3	1	2	2	4
93	96-232-27	SeqSNP	3	3	0	0	1	1	1	2	1	1	1	1	2	2	1	2	2	1	2	1	1	3	3
93	96-232-27	KASP	3	3	0	0	1	1	1	2	1	1	1	1	2	2	1	2	2	1	2	1	1	3	3
95	Abnaki	SeqSNP	1	1	0	1	2	0	0	2	2	0	2	1	2	2	3	0	3	2	2	2	2	2	4
106	Accent	SeqSNP	2	0	2	2	2	2	3	3	4	1	2	2	2	2	2	4	2	2	2	3	1	2	2
192	Adato	SeqSNP	3	3	2	3	4	4	3	0	1	3	1	1	1	1	1	1	4	1	1	3	1	4	3
192	Adato	KASP	3	3	2	3	4	4	3	0	1	3	1	1	1	1	1	1	4	1	1	3	1	4	3
99	Advira	SeqSNP	3	1	3	1	2	1	3	3	4	2	3	4	2	1	1	2	2	2	2	2	3	3	1
102	Agatha 11	SeqSNP	1	2	0	4	2	1	3	2	3	1	0	2	4	1	2	0	4	1	1	1	4	1	1
19	Agria	SeqSNP	2	1	2	2	3	4	3	3	2	2	3	1	1	2	3	2	2	3	1	2	3	3	2

Figure 6. A segment of the potato SNP genotype database as determined with two different genotyping methods (SeqSNP and KASP) at 23 SNP positions.

Breaking the cycle of poverty: Empowering vulnerable women in an informal settlement with food cultivation skills

Compiled by Sunette Laurie, Igenicious Hlerema, Erika van den Heever, Manaka Makgato and Nadia Araya

The global Multi-dimensional Poverty Index (MPI) describes poverty as a lack of basic resources such as health, nutrition, hygiene, assets, living standards and education, and all possible barriers, or contributors to a good and healthy life. A collaborative project is run by the ARC and the University of Pretoria to capacitate 25 vulnerable women from the Cemetery View Informal Settlement (Woodlands, Pretoria), with skills towards economic independence, food security and improved personal health. On a weekly basis, the ARC presented several horticultural topics with the focus on space-efficient vegetable production as part of the establishment of the Imvelo Food Demonstration Garden at the Living Word Premises neighbouring the community (Fig. 1). Training included seedling production, preparing and planting of the bag systems, growing Moringa, preparing bags for mushroom production using oyster mushroom spawn (Fig. 2), and the making and planting of containers with high beta-carotene tomatoes. Vertical production can yield 12 kg of leafy vegetables from 40 plants per bag and provides four harvests over a 2-3 month growing period.

Open field production was challenging, since it is an old building rubble dump site, but the community managed to cultivate the land after rain, and a plot of orange-fleshed sweet potatoes was established (Fig. 3). Orange-fleshed sweet potatoes are particularly rich in beta-carotene. A 70 g portion provides the recommended daily allowance of vitamin A for preschool children.

Additional activities from collaborators included cooking, demonstrations, healthy eating, life skills, as well as setting up a poultry house for egg production. This project brought together qualified professionals and experts in primary health care, dietetics and nutrition, food cultivation, and animal husbandry. The combination of these disciplines for targeted health and nutritional skills transfer will have a significant impact on vulnerable women living in the Cemetery View informal settlement.

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Figure 1. Women being trained at the Imvelo Food Garden in the planting and watering of vegetables in maize meal bags. The Cemetery View community is visible at the back.

Figure 2. Filling bags with wheat straw for germination of oyster mushroom spawn for home production.

Figure 3. Making ridges and plant orange-fleshed sweet potatoes.

Capacity development of mothers in growing orange-fleshed sweet potato in the Eastern Cape

Compiled by Musa Mtileni and Sunette Laurie

The ARC, in partnership with the Department of Social Development, Department of Health, Department of Rural Development and Agrarian Reform, and Old Mutual are implementing a project promoting the production of orange fleshed sweet potato (OFSP) as part of the Integrated Mother and Child Development and Support Programme (IMCDSP). This is an intervention programme that is fighting child poverty and malnutrition. The programme is an immediate response to the increase in the incidence of infant mortality in the OR Tambo and Alfred Nzo districts, and is aimed at empowering women with children below the age of five. The OFSP Project was launched on 12 August 2021 as part of the Women's Month Programme for 2021.

Capacity development of mothers was conducted over the period 16 – 24 November 2021, and is being piloted in three local municipalities of Ingquza Hill, Port St Johns and Ntabankulu, in the Eastern Cape Province. A total of 101 women, including young mothers, were trained during theoretical sessions on: How to plan a vegetable garden, Production practices of sweet potato, and Benefits of orange-fleshed sweet potato. Practical demonstrations were performed on plots of 8 m x 6 m in size and included other pro-vitamin A rich crops such as Swiss Chard, carrot and butternut, in addition to OFSP. The practical sessions included plot demarcation, land preparation, fertilizer application, ridge making, plant spacing, handling of propagation material and crop management. After the practical session each mother who attended the training received twenty cuttings of OFSP to plant in their home gardens.

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One of the theoretical training sessions at Ntabankulu.

Practical demonstration of planting sweet potato cuttings at Port St Johns.

Plot demarcation and preparation.

Mulch applied during a demonstration session at Lusikisiki.

Technology Transfer - October to November 2021

Scientific publications

Cruywagen, E.M., Pierneef, R.E., Chauke, K.A., Nkosi, B.Z., Labeda, D.P. & Cloete, M. 2021. Major *Streptomyces* species associated with fissure scab of potato in South Africa, including description of *Streptomyces solaniscabiei* sp. Nov. *Antonie van Leeuwenhoek* 114:2033–2046 https://doi.org/10.1007/s10482-021-01659-8

Maphothoma, P., Kleynhans, R., Prinsloo, G., Mokgehle, S.N., du Plooy, I. & Araya, H.T. 2021. Growth and yield of African ginger in response to application of organic fertiliser. *South African Journal of Plant and Soil* 38(4): 338–342

Maponya, P., Oelofse, D., Madakadze, I.C., Mbili, N., Dube, Z.P., Nkuna, T., Makhwedzhana, M., Tahulela, T., & Mongwaketsi, K. 2021. Socio economic status and agroforestry readiness: A case study of selected communities in the OR Tambo District, Eastern Cape in South Africa, *AGROFOR International Journal*, Issue 2, Volume 6, 68 – 76, November 2021.

Scientific publications

Phahlane, C.J., Laurie, S.M., Shoko, T., Manhivi, V.E. & Sivakumar, D. 2021. An evaluation of phenolic compounds, carotenoids, and antioxidant properties in leaves of South Africa cultivars, Peruvian 199062.1 and USA's Beauregard. *Front. Nutr.* 8:773550. doi: 10.3389/fnut.2021.773550

Selokela, L.M., Laurie, S.M. & Sivakumar, D. 2021. Impact of different postharvest thermal processing on changes in antioxidant constituents, activity and nutritional compounds in sweet potato with varying flesh colour. *South African Journal of Botany* 144: 380-388

Post-graduate degrees

Masondo, S.N. 2021. Effect of plant growth substances and nitrogen application on growth, yield and quality parameters of Swiss chard (*Beta vulgaris* L.). Master Technologiae (Agriculture), Tshwane University of Technology

Did you know?

The ARC is celebrating 70 years of sweet potato breeding in 2022!

Breeding of sweet potato started in 1952 at the Roodeplaat campus and since then a large number of cultivars (31) have been released. In those initial years, farmer selections such as Virovsky, Hoenderspoor, Borrie, Three Months White and Six Months White were produced. But these produced crooked shaped tubers and defects such as veins, grooves and cracks, therefore, there was a clear need for formal breeding. Hand crosses were made among these farmer's varieties and with sweet potato germplasm imported from the USA (Louisiana and South Carolina). Mafutha was the first cultivar released in 1959, followed by six more cultivars released during the 1960's (Impala, Wildebees, Griekwa, Eland, Hartebees and Koedoe) and another four until the 1980s, including Ribbok and Bosbok, which are still grown today.

In the 1980's, the polycross system was adopted to improve crossing efficiency and a large number of multi-location cultivar evaluations were conducted. This led to the release of Blesbok in 1989, which still today is the major sweet potato cultivar produced in South Africa.

As from 1995, the needs of small-scale farmers and nutrition security became the major aim of the program. Between 2003 and 2013, 14 sweet potato cultivars were released, of which seven were β-carotene enriched and nine were improvements of Mafutha (sweet taste and drier texture with improved yield and storability). Of these, the most popular informal market cultivars are Ndou and Monate (cream, dry), and orange-fleshed Bophelo. The latest addition is Khumo (meaning richness), an OFSP released in 2020.

We salute Mr Andre van den Berg (below), sweet potato Senior Research Technician, for 43 years of service to the ARC for his immense contribution to the sweet potato breeding program.